differences in DNA structure within tumor initiation and tumor promotion sites. Right-handed double-stranded (ds-) B-DNA is the conventional structure of DNA that results in the majority of DNA. As part of our past work we examined the epidermis of normal human skin for the presence of ds-B-DNA as it undergoes destruction due to normal cell death processes [i.e., apoptosis and terminal differentiation (denucleation)]. Our research team has examined the distribution and intensity of anti-B-DNA antibody binding in human melanoma; formalin-fixed paraffin-embedded tissue sections (1 micron). We also employed a variety of different anti-melanoma antibody probes [e.g., (HMB45) (ab787)]. Using enhanced histotechnological processing procedures we were able to better preserve the melanoma tissue-bound B-DNA (i.e., intact, unaltered and non-denatured nucleic acids). Superior preservation of tissue-bound components resulted in improved characterization of the immunostaining data (1). We characterized the lateral and vertical margins of the epidermal tumor growth to see if any changes were occurring in the non-cancerous areas of the epidermis next to the tumor sites. We found that B-DNA is located in all cells, and that the binding intensity [mean optical density] of immunohistochemistry is similar in all regions of the cancerous growth. Being able to locate hyperactive regions of B-DNA in the tumor growth will allow for new drug target sites. disease. 1. Gagna C.E., et al., (2007) Novel DNA Staining Method and Processing Technique for the Quantification of Undamaged Double-Stranded DNA in Epidermal Tissue Sections by PicoGreen Probe Staining and Microspectrophotometry. Journal of Histochemistry and Cytochemistry. 55: 999-1014. Supported by a NYIT-ISRC grant.

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Double-Stranded B-DNA: Presence in Human Melanoma Tissue (Stage III)

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Stage III melanoma refers to tissue tumors that have spread to regional lymph nodes, or have developed in transit metastasis (regional). With treatment this pathology is considered intermediate to high risk for recurrence locally or for distant metastasis. Consequently, new approaches towards treating melanoma need to be developed. Right-handed double-stranded (ds-) B-DNA is the most common structure that makes up the majority of DNA. Tissue samples were preserved in several different tissue fixatives (molecular grade), in order to better characterize DNA and DNA-protein complex interactions (10% formalin, 10% neutral buffered formalin, Clarke's solution, Carnoy's, solution, and zinc formalin fixative). Previously, we have characterized the epidermis of human skin for the presence of ds-B-DNA as it undergoes cell death [i.e., apoptosis and terminal differentiation]. Our data reveals the distribution and intensity of anti-B-DNA, anti-single-stranded DNA, anti-Z-RNA antibody binding, and a variety of different anti-melanoma antibody, in human melanoma (IIIA, IIIB, and IIIC). We carefully observed the differences in DNA structure within the papillary dermis. The intensity of immunohistochemical staining is different within certain regions of the cancerous growth, namely, less immunohistochemical staining in the lateral regional and much more in the vertical areas. Less ss-DNA was seen in the vertical areas (reticular dermis). Employing novel histotechnological processing procedures we were able to better preserve the tissue-bound ds-B-DNA as intact, unaltered and non-denatured molecules (1). This has resulted in improvements involving laser capture dissection techniques for the isolation of genetic materials. 1. Gagna CE, et al., (2007) Novel DNA Staining Method and Processing Technique for the Quantification of Undamaged Double-Stranded DNA in Epidermal Tissue Sections by PicoGreen Probe Staining and Microspectrophotometry. Journal of Histochemistry and Cytochemistry. 55: 999-1014. This research project was supported in part by a 2011 ISRC grant.

372-Pos Board B127

Intact Right-Handed B-DNA: Occurrence in Human Melanoma Tissue (Stage I)

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Medicinal treatments for this pathology are limited and therefore new approaches need to be taken, namely, new classes of drugs and/or biologicals. Tissues were processed in formalin-fixed and non-formalin-fixed paraffin-embedded tissue sections. Proper fixation of melanoma tissue samples is critical for the correct preservation of tissue morphology, and especially tissue-bound nucleic acids. Improper fixation will lead to regions of single-stranded (ss-) DNA that can interfere with the correct characterization of the tissue-bound components. Our group has developed a histotechnological procedure to preserve undamaged nucleic acids (1). Right-handed double-stranded (ds-) B-DNA is the conventional structure of DNA. In the past we have examined the epidermis of normal human skin for the presence of ds-B-DNA, ds-Z-DNA as it undergoes destruction due to the normal process of cell death [apoptosis and terminal differentiation (denucleation)]. Our research team has examined the distribution and intensity of anti-B-DNA antibody and anti-melanoma antibody binding in human melanoma (Ia and I B). Our results show that B-DNA is located in all cells of the melanoma tissue; however, the intensity of immunohistochemical staining is different within certain regions of the cancerous growth (an increased amount of ds-DNA content the vertical growth phase zone: papillary dermis). Using enhanced histotechnological processing procedures we were able to better preserve the tissue-bound ds-B-DNA, which was not damaged from tissue processing (i.e., ds-DNA converting to ss-DNA). This resulted in intact ds-B-DNA. Being able to locate intact ds-B-DNA in the cells of cancer will allow for the identification of specific target sites. 1. Gagna C.E., et al., (2007) Novel DNA Staining Method and Processing Technique for the Quantification of Undamaged Double-Stranded DNA in Epidermal Tissue Sections by PicoGreen Probe Staining and Microspectrophotometry. Journal of Histochemistry and Cytochemistry. 55: 999-1014. Supported by a 2011 ISRC grant.

373-Pos Board B128

Unaltered B-DNA: Distribution in Human Melanoma Tissue (Stage II)

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Stage II melanoma is a localized tissue tumor. Proper fixation of melanoma tissue samples is extremely important for the accurate preservation of tissue morphology, and mainly nucleic acids. Incorrect histotechnological fixation will lead to regions of denatured single-stranded DNA that can interfere with the correct characterization of the nucleic acid tissue-bound components. We have developed a procedure to preserve intact nucleic acids (1). Right-handed double-stranded (ds-) B-DNA is the conventional structure of DNA. Our team has examined the epidermis of normal human skin for the presence of ds-B-DNA as it undergoes cell death [apoptosis and terminal differentiation]. Our group has now examined the distribution and intensity of anti-B-DNA antibody, anti-melanoma antibody, and anti-single-stranded (ss-) DNA binding in Stage II human melanoma. Our results show that B-DNA is located in all cells of the melanoma tissue; however, the intensity of immunohistochemical staining is different within certain regions of the cancerous growth. Less immunohistochemical staining was found in the lateral regional and more in the vertical areas (i.e., papillary dermis). Using enhanced histotechnological processing procedures we were able to better preserve the tissue-bound ds-B-DNA. Consequently, the DNA was not damaged from tissue processing, and did not result in denatured ss-DNA that would interfere with the characterization of ds-B-DNA. We are also looking at differences between the DNA of ulcerated and non-ulcerated melanomas. Being able to differentiate between ds-B-DNA and ss-DNA in the cancer tissue will allow for the identification of specific nucleic acid target sites. 1. Gagna C.E., et al., (2007) Novel DNA Staining Method and Processing Technique for the Quantification of Undamaged Double-Stranded DNA in Epidermal Tissue Sections by PicoGreen Probe Staining and Microspectrophotometry. Journal of Histochemistry and Cytochemistry. 55: 999-1014. Supported by an ISRC grant.

Protein-Nucleic Acid Interactions I

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The Mechanism of Nucleosome Spacing by a Dimeric Chromatin Remodeling Enzyme

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Transcriptional regulation and cellular differentiation involve changes in the structure, composition and post-translational modification state of chromatin, the material that packages the eukaryotic genome. Changes to chromatin structure require the action of chromatin remodeling enzymes, protein complexes that use the energy of ATP hydrolysis to move, disassemble or deposit nucleosomes. Despite their central importance to chromatin biology, the mechanism by which chromatin remodelers break histone-DNA contacts and reposition